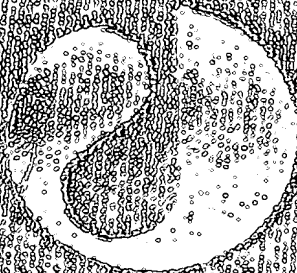


10/677,956/Declaration, Torston B. Holling

January 7, 2007

EXHIBIT# 4



Pharmacia

Pharmacia Genetic Engineering Inc.

NO. 51

6.29 Cloning of Capsid PCR fragments

PCR reactions of 6.28 #1 690-691 360 bp full size
all from SF.cDNA 190 bp ?

#3 690-694 233 bp

#5 891-693 215 bp

set up remainder of PCR reaction on 5% PAGE prep gel
1 day \Rightarrow EtBr stain - cut out above bands

4 bands coil gel slice - electroelute 15' 1 watt
20' 3 watts

collect DNA

ϕ -CHCl₃ extract

EtOH ppt -80° 1 hr

spin out DNA

set up kinase reactions

pellet up in 76 μ l H₂O

2 μ l 1M ATP

2 μ l PPK

10 μ l link kinase

37° 1 hr

ϕ -CHCl₃ extract

EtOH ppt -80° 1 hr

pellet up in 20 μ l H₂O

1/2 of pellet amt into ligation reactions

6.5 μ l H₂O

10 μ l DNA

0.5 μ l PCR small cut

2 μ l 10x ligase kinase buffer

1 μ l T₄ ligase

rt o/n

6.30 Transformation

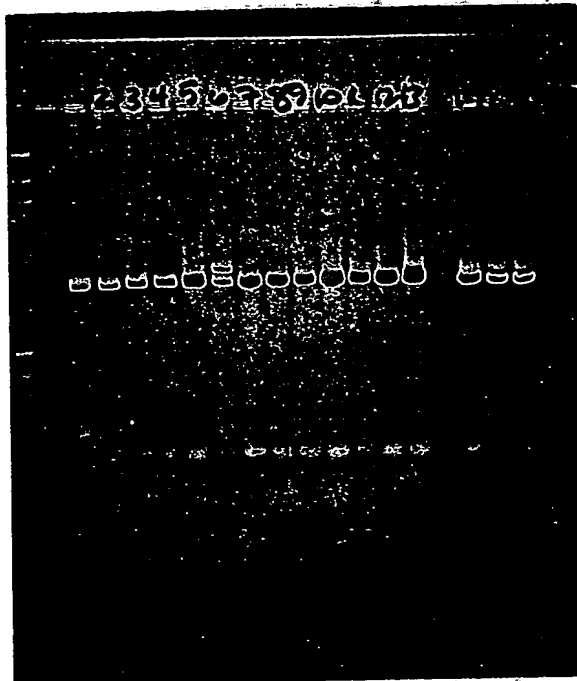
IMB3 cells 4 x PCR-PCR reactions

plate cells on XGAMP plates

7.1 replate as not many x-formants

7.2 re-transform ligations - IMB3 to increase #'s

BEST AVAILABLE COPY



7.4

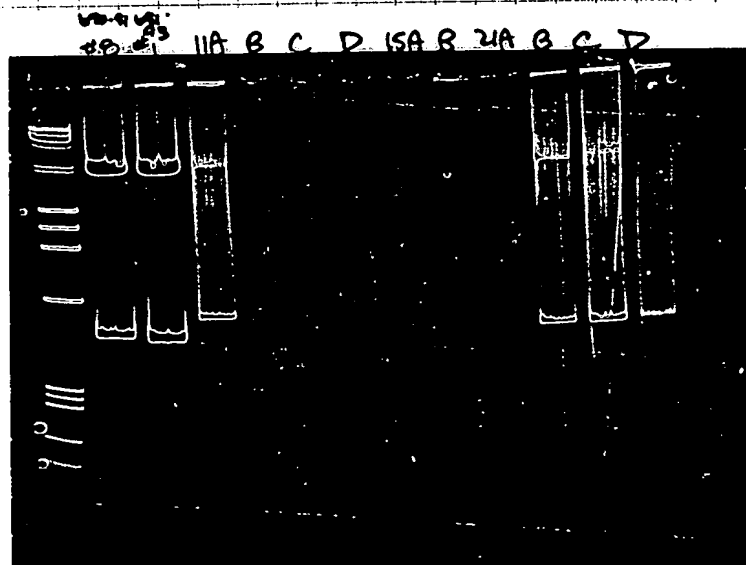
PHOTOMETRY/DNASCAN4U

07/09/90 11:01

ID ABS

260.0nm	0.209A	691473 #1	1.045
260.0nm	0.146A	69106911 #8	0.73

260	0.391	690694 #9
-----	-------	-----------



7.9

7.1 mini preps on capsid fragments into PCR 18

CYNDIES preps

PCR 1 top = mprep #1 → 3 690.691
 PCR 1 btm = mprep #4 → 9 690.691
 PCR 3 = mprep #10 → 18 690.694

see net notebook for results

→ only positive = PCR 1 btm #9
 690.691

7.2 more mini preps

PCR 1 top = #1 → 3 690.691
 PCR 5 = #1 → 13 691.693

see photo at right

→ only positive = PCR 5 #1
 691.693

7.4 more mini preps

#1 → 9 PCR 1 top 690.691
 #1 → 9 PCR 3 690.694

see cyndies notebook -
 identified positive
 PCR 3 690.694 #9

7.5 maxi preps on

690.691 btm #8
 691.693 (PCR 5) #1

7.9 maxi prep on

690.694 #9

7.8 mini preps repeated on cyndies of 7.7 →

positive 690.691 #15
 #21
 690.694 #11

but colonies = double pick
 replat XGAMP (all white)
 choose separates:
 remini prep. (A → D and

PvuII digest 4 x mpreps of each (ISC+D did not grow)

choose 11A 690.694
 213 690.691

7.10 maxi prep

ABS

260.0nm 0.156A 11A 780ng/ul
 260.0nm 0.391A 213 1.955ug/ul

7.13 remini prep #15 690.691 isolates → EFGH1J

see CYNDIES notebook

choose 2 for maxi prep

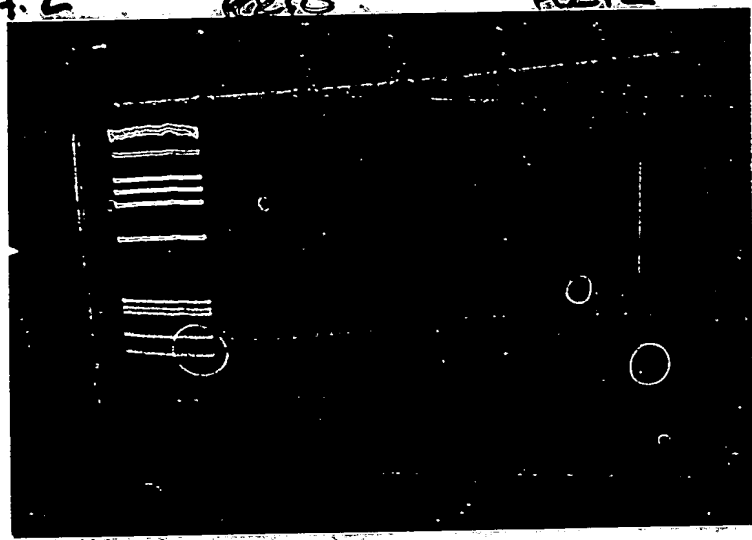
7.16 maxi prep of #15 E : 15H 690.691

Page 67 = continuation of
 cloning attempts

7.2

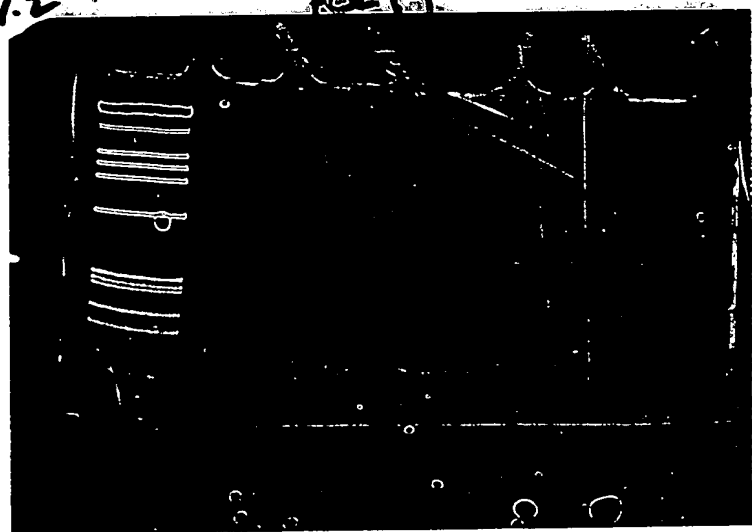
REC 10

REC 12



7.2

REC 14



7.2 Cloning of PCR fragments from 687-688 oligos 0000

PCR 10 687-688 fragments of 230 bp (1. than predicted 117 bp)
 PCR 12 682-687 250 bp fragment (not as predicted)
 PCR 14 683-687 150 bp fragment (smaller than predicted)

run remainder of PCR reaction on 5% PAGE prep gels

cut out ↑ bands 230 bp PCR 10
 250 bp PCR 12
 150 bp PCR 14

elute 20 1 watt
 20 3 watt } CHCl₃ extract
 EtOH ppt -80° 1 hr

kinase 2ul 1M ATP
 2ul Pfu
 10ul 10x linc-kin buffer
 70ul H₂O to pellets

37° 1/2 hr CHCl₃ extract
 -80° 1 hr EtOH ppt res. up in 20ul
 ↳ freeze 1/2 -20

ligate 10 PCR 10

10ul DNA
 6.5ul H₂O
 0.5ul Pfu small
 2ul 10x ligase kinase buffer
 1ul T4 ligase

part

7.3 Transformation into JM83 cells plate onto Xba Amp plates

Results show no whites ∴ few colonies NO colonies for PCR 14

18 replate transformations to increase colony numbers

7.9 minipreps on PCR 10 PCR 12
 #1→9 #1→9 } Pvu II digested
 → SEC CYANIDE NB

results show no positive colonies

7.12 repeat of more PCR 10 PCR 12 preps

again no positive colonies ⇒ redesign clones!

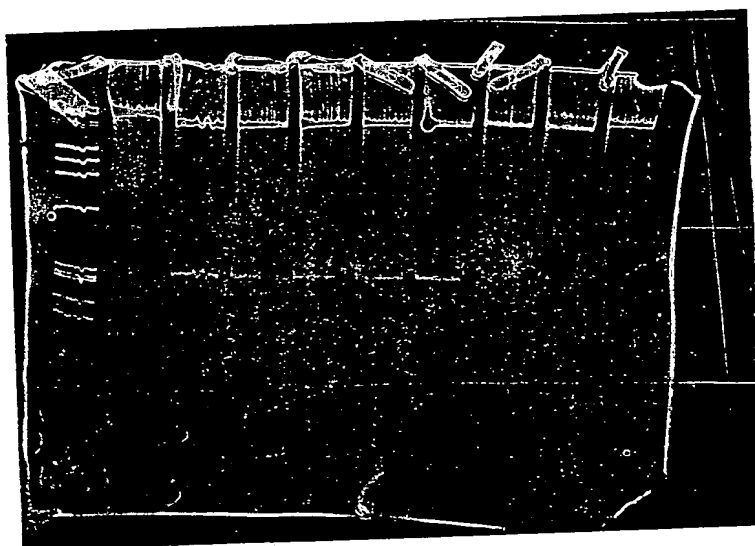
Attempts to reisolate 693-691 clones

only one previously identified
691-693-#1

sequence analysis (p 79) shows shorter than
expected - repeat from ligation to
identify more clones

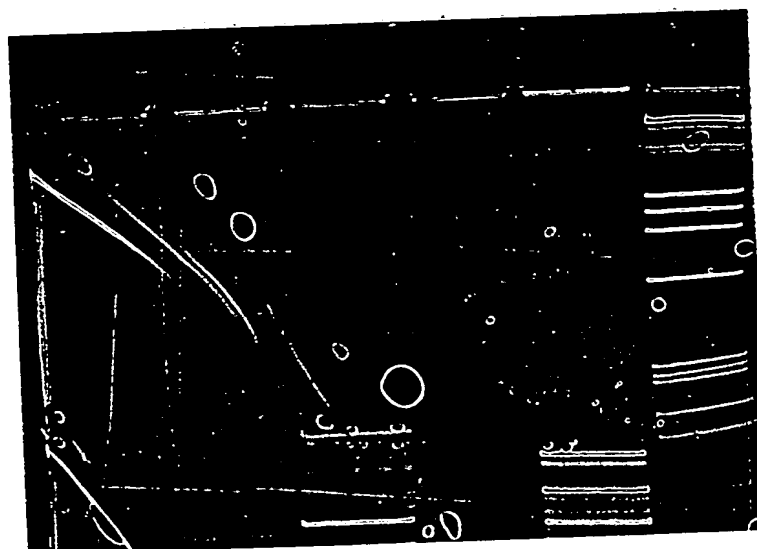
SEC CINDIES Notebook for xformations
mini preps

Attempts RVI digests of mpreps



7-19 CYNs MPREPS

no positives identified



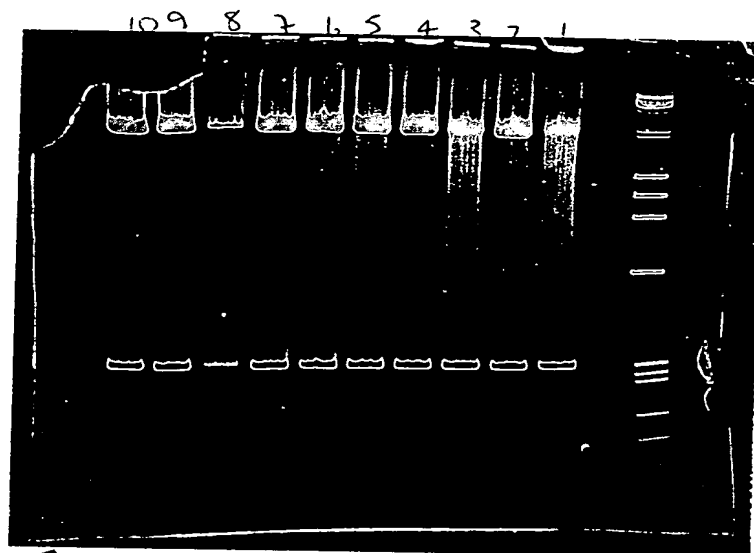
7-25

only PRS.1
good 5

7.23

more 691693 mpreps
 replate PCR 5 xformation mix
 choose white colonies
 grow white clones → in Amp
 duplicate to lower more mprep
 Puvi digest

Results



7.25

7.24 Repeat PCR clonings of 690691 Full capsid = 691693

Use 1st round PCR from 6.27 - page 63

*1-14 stored at -20°

resetup #1 690691 S9 cDNA
 #5 691693 S9 cDNA
 #6 691693 PCR cDNA

new PCR's

		AB	TAQ	H ₂ O	690	691	693	1
1	*1 redo	50	10	256	2.76	1.61	-	-
2								
3								
4								
5	*5 redo			26.69				17
6								
7	*6 redo			26.69				
8								
9								
10								

results
 ←

EVE FINE 11

① CHCl₃ ECH apt up in 400ul H₂O → prep gel

35S reactions

626.627	590 BS primer	
626.627	591 BS primer	
626.627	626 primer	
626.627	627 primer	
628.629	628 primer	
628.629	652 primer	
2.F.	674 primer	674.675 = 2.F.
2.F.	675 primer	
571.623 #10	571 primer	

Results show priming with all -

590/591 = shadow bands

626 = light sequence

others look good

7.3 35S sequences entered into VAX

200-300 bp. for each combo.

Processing data of 30JUN90 ALF.

- 1] processed ok, but all overlapping peaks
- 2]
- 3] processed ok, but overlapping peaks
- 4]
- 5 processed - 225+ bases - weird pattern : can continue
- 6 could not compute peak width. Given peaks ? - off scale?
- 7 processed - 240 bases
- 8 peak width off - could not process
- 9 processed, but overlap peaks
- 10 could not compute peak width.

peaks w/ reverse primer.

62790

35S sequencing of HepC clones - use internal primers.

To Denature					5'ug
626, 627 (#46)	4 tubes	1ul = 1.21ug		4.13	
628, 629 (#16)	2	1ul = 1.56ug		3.2	
2, F. (#39)	2	1ul = 0.43ug		11.6	
571, 623 (#10)	1	1ul = 1.98ug		2.5	

To each tube add 2ul 2N NaOH.

leave rt 10'

+ 3ul NAC

+ 100ul EtOH + 20ul H₂O -70° 1hr wash

Follow Cynclis procedure using Pharmacia kit.

run on 8% Gels.

short = BBOff

long = XCoff + 3/4 of 2nd r

62990

ALF sequencing to complete clone information.

PCR 2#2	1ul = 0.465ug
PCR 2#7	1ul = 0.74ug
626, 627	1ul = 1.21ug
628, 629	1ul = 1.56ug

302uN ALF

(problems w/ 29 JUN 90 ALF)

#			QNA	H ₂ O
1	PCR 2 #2	universal	10	-
2	PCR 2 #2	reverse	10	-
3	PCR 2 #7	universal	6.75	3.25
4	PCR 2 #7	reverse	6.75	3.25
5	626, 627	universal	4.13	7.87
6	626, 627	reverse	4.13	7.87
7	628, 629	universal	3.2	6.8
8	628, 629	reverse	3.2	6.8
9	PCR 2 #2	universal	10	-
10	PCR 2 #2	reverse	10	-

resulting reported sequences = only 2!

626, 627 - U. Seq

628, 629 - U. Seq

571623 clone

571623-571.Seq Length: 294 July 3, 1990 15:53 Check: 3145

```
1 AGTGGGGAGA ACTTTCCTTA CCGTAGCG TACCAAGCCG CCGTGTGCGC
51 TAGGGCTCAA GCCTCCCCCA TCGGGGACC AGATGTGAA GTGTTGATC
101 CGCTTAAAC CCACCTCCA TGGGCCAAC ACCCTGGAT ACAGACTGGG
151 CGCTGTTCAG AATGAAGTCA CCGTACGCA GGGAAACAC #AATACATCA
201 TGACATGCGG TACCGAGCTC GAATTCGTAA TCAATGATA GCTGTTCTT
251 GTGTGAATTG TTATCGCTC ACAATCCAC ACAACATAC AGCC
```

571623-10f.Seq x 571623-571.Seq July 3, 1990 10:30 ..

```
74 AGTGGGGAGA ACTTTCCTTA CCGTAGCG TACCAAGCC ACCGTGTGCGC 123
|||||
1 AGTGGGGAGA ACTTTCCTTA CCGTAGCG TACCAAGCC GCCGTGTGCGC 50
124 TAGGGCTCAA GCGCTCCCCA TCGGGGACC AGATGTGAA GTGTTGATC 173
|||||
51 TAGGGCTCAA GCGCTCCCCA TCGGGGACC AGATGTGAA GTGTTGATC 98
174 TCCGCTTAA ACCCACCTT CCAATGCG TACCAAGCC ACCGTGTGCGC 222
|||||
99 TCCGCTTAA ACCCACCTT CCAATGCG TACCAAGCC ACCGTGTGCGC 147
223 GGGCGCTGT TCAAGTGA AGTCAACCT TCAAGTGA AGTCAACCT 272
|||||
148 GGGCGCTGT TCAAGTGA AGTCAACCT TCAAGTGA AGTCAACCT 197
273 TCAATGATA GCTGTTCTT
|||||
198 TCAATGATA GCTGTTCTT 208
```

see page 37

571623 clone info

- confirms seq. A.

alterations from previous seq's.

21f1-U.Seq x 21f1-675.Seq July 3, 1990 10:35 ..

```
134 agcgagcgggtggttcctcaccgaatcaaccctacctaactgaccttgggcaag 183
|||||
1 AACGTACGCTGTGCTCCACCGAATCAACCTTACCTACTGCTTGGCGAG 49
184 ctgcccaccccaagtttgccagctcctcaacttccggcattacgggca 237
|||||
50 CTGCGCACCAAAAGTGTGCGAGCTCCCTCAACTTCCGCGATTACGGGCGA 99
|||||
234 caatacgacaacatcctctgagcccgcccttctggtg.ccccccgaact 282
|||||
100 CAATACGACAACATCTCTGAGCCCGCCCTTCTGCTGCCCCCGGACT 149
283 ccgagcttgagtcctattcttccatgccccccctgggggggacctgggga 332
|||||
150 CCGAGCTTGAGTCTTATCTTCCATGCCCCCTTGGAGGGAGCTGGGGGA 199
333 t.cgggtctcagcgagcgggtcctggtcgagcgggtcagtagtggggc 376
|||||
200 TCCGATCTCAGCGACGGGTCAATGCTGACGGTCAATAGTGGGGC 244
```

SEQUENCE ANALYSIS

50 JUN 90 ALF

3'S sequences

* what was in computer for.

ZIFI clone from NYBC. - page 59

1 GTTGCAGTCT GTCAAATGTG ACTTTCTTCT GCCTTTGNAG CACTGCCTGA

355
result

151 CGTTCCAGGA ATAAGACAAT GAGCAGCACA CGACATCCTC CGTGTGGGCC

251 CTCCCCCTCC AGGGGGGCACC TCCAAGAATA GGAC

1 AACGTACGGT GGTCCTCACC GAATCAACCC TACCTACTGC CTTGCCGAGC

355
result

151 CGACGTTGAG TCCTATTCTT CCATGCCCCC CCTCGAGGGA GCCCTGGGGAT

201 CCGGATCTCA GCGACGGGTC ATGGTCGACG GTCAGTAGTG GGGCCGACAC

251 GGAGGTGTCTG TGTGCTGCTC AATGTCCTTAT TCTGGACAGG CGCACTCGTC

301 AC

160 GTCCTATTCTTCCATGCCCCCTGGA..GGGAGCCTGGGGATCCGGATC 207

||||| | ||||| |||||
302 GTCCTATTCTTGGAGGTTGCCCCCTGGAGGGGGAGCCTGGGGATCCGGATC 253

208 TCAGCGACGGGTCATGGTCGACGGTCAGTAGTGGGGCCGACACGGAGG^A.T 256

252 TCAGCGACGGGTCATGGTCGACGGTCAGTAGTGGGGCCGACACGGAGGAT 203

257 GTCGGTGTGCTGCTCAATGTCTTATTCTGGACAGGCGCACTCGTCAC 302

202 GTCGTGTGCTGCTCAATGTCTTATTCCTGGAAAGGGCGCACTCGTCAC 156

matches.
↓
work

119 ttgcagtcctgtc ^Aaatgtgactttcttctgcctttg ^{OK}gcagcactgcgtg 167

2 TTGCAGTCTGTCAAATGTGACTTTCTTCTGCCCTTG..NAGCACTGCCTG 49

168 aagtggtggaatacaccagattgtgatggccta 200

50 AAGTGGTGG AATACACCAGATTGTGATGGCGTA 82

to previous
xq
4-

628629-U.Seq x 628629-628.Seq July 5, 1990 11:03 ..

628629-628.Seq x 628629-R.Seq July 6, 1990 07:45 ..

628629-U.Seq x 628629-R.Seq July 5, 1990 11:02 ..

cannot identify homology with 652 sequence
link up other seqs into 628/629 - F. seq.

2590

000

Sequence Analysis of 628629 clone

from 355 sequence - 303W ALF

628629-U.Seq Length: 225 July 2, 1990 11:34 Check: 8218 ..

1 TAATACGACT CACTATAGGG CGAATTGGGT ACCGGGCCCC CCGTCGAGGT
 51 CGACGGTATC GATAAGCTTG ATATCGAATT CCTGCAGCCC TCGTTGTGGA
 101 TGATGCTACT CATATCCCAA GCGGAGGCGG CTTTGGAGAA CCTCGTAATA
 151 CTCAATGCAG CATCCCTGGC CCGGACGCAC GGTTTTGTGT CTTTCTCTGT
 201 GTTCTTCTGT TTTCGCTGGT ATCTG

ALF

628629-652.Seq Length: 247 July 3, 1990 15:42 Check: 6340 ..

1 CATTCGTAT TACGAGGTTT TCCAAAGCCG CCTCCGCTTG GGATATGAGT
 51 AGCATCATCC ACAAGCAGGG CTGCAGGAAT TCGATATCAA GTTATCGAT
 101 ACCGTGACCC TCGAGGGGGG GCGCGTACC CAATTCGCCC TATAGTGAAT
 151 GGTATTACGC GCGCTCAGTG GCGCTCCTTT TACAACGTGG TGAATGGGAA
 201 AACCCTGGCG TTACCCAACI TAATCGCCTT GCAGCACATC CCGCTTT

355

628629-628.Seq Length: 265 July 3, 1990 15:39 Check: 3747 ..

1 GTTGGAGAA CCTCGTAATA CTCAATGCAG CATCCCTGGC CCGGACGCAC
 51 GGTTTTGTGT CTTTCTCTGT GTTCTTCTGT TTTCGCTGGT ATCTGAAGGG
 101 TAGGTGGGTG CCCGGAGCGG TCTACGCCCT CTACGGGATG TGGCTCTCC
 151 TCTGTCTCT GTTGGGCTTG CCTCAGCGGG CATACGCACT GGACACGGAG
 201 GTGGCCGCGT CGTGTGGGNG CTTTGTCTTT GTGGGTTAA TGGGCTGAC
 251 TCTGTGCGCA TATTA

355

Previous Sequence Results page 58

628629-1

628629-N48C

Bestfit Analysis

626627-590.Seq x 626627-626.Seq July 5, 1990 15:32 ..

165 ctgagctcgggacctgtcctgtcttccagatcacagcttt 203
 1 CTGAGCTCGGACCTGTCTGTCTTCCAGATCACAGCGTT 39

b26?

626627-U.Seq x 626627-591.Seq July 6, 1990 08:13 ..

72 TATCGAATTCCTGCGACCCCGCAAAGAGCGTGTGTGGCCCGGTATATTG 121
 1 TATCGAATTCCTGCGACCCCGCAAAGAGCGTGTGTGGCCCGGTATATTG 50
 122 CTTCACCTCCCGACCCCGTGGTGGTGGGAACGACGAGGTGCGCGCCTA 171
 51 CTTCACCTCCCGACCCCGTGGTGGTGGGAACGACGAGCA.GTGGCGCGCCTA 99
 172 CCTACAGCTGGGGTGCAAAATGATACGGATGTCTTCGTCTTAAACAACAC 220
 100 CCTACAGCTGGGGTGCAAAATGATACGGATGTCTTCGTCTTACACAACAC 149
 221 CAGGC 225
 150 CAGGC 154

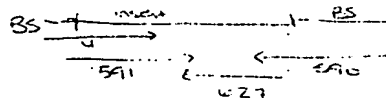
626627-U.Seq x 626627-Nybc.Seq July 5, 1990 15:55 ..

91 CCGCAAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCGACCCCGTG 140
 1 CCGCAAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCGACCCCGTG 50
 141 GTGGTGGGAACGACCGACAGGt...GCGCGCCTACCTACAGCTGGGGTGC 187
 51 GTGGTGGGAACGACCGACAGGTCGGGCGCGCTACCTACAGCTGGGGTGC 100
 188 AAATGATACGGATGTCTTCGTCTTAAACAACACACAGGC 225
 101 AAATGATACGATGTCTTCGTCTTAAACAACACACAGGC 138

626627-Nybc.Seq x 626627-591.Seq July 6, 1990 08:15 ..

276 GNTATTTANGGAAGCAATCAGTGGGGCAGAKCAAGGTGTGTGTGGCGN
 299 GATATTGGG.AAGCAATCAGTGGGGCAGAGCAAGGTTGTGTGGCCCA
 226 CCTCCGATGACNCAAGGGGGNGCTCCGACACTTGTGTGAATCCAGT
 250 CCTCCGATGACACAAGGGGGCG.TCCGACACTTGTGTGAATCCAGT
 176 GTTCATCCAGGTACAACCGAATCAATTGCCAGCGGTGGCTTGGTGT
 201 GTTCATCCAGGTACAACCGAATCAATTGCCAGCGGTGGCTTGGTGT
 126 .TAAAGGAYGAAGACATTCATTTGCACCCAGCTGTAGGTAGG
 151 GTAAGGACGAAGACATCCGATATTCATTCACCCAGCTGTAGGTAGG
 77 GCGGACCTGTGGTTCGTTCCACACACAGGGGCTGGAGTGAAGC/
 101 GC....ACTGTGGTTCGTTCCACACACAGGGGCTGGAGTGAAGC/
 27 ATACCGGGCCACACAGCTCTTTGCGG 1
 55 ATACCGGGCCACACAGCTCTTTGCGG 39

Organization



7-6-90

Construction/organization of sequence information

0000

626627 clone from NYBC

626627-590.Seq Length: 203 July 3, 1990 15:20 Check: 760 ..

1 actaaagggg acaaaagctg gagctccacc gcggtgcgcc gctctagaac
 51 tagtggatcc cccaatgttc tggtaggagt ggaatgaggcc ggtggacaag
 101 gctggcaggg tcgtgaaaga ccacgggaag acctgccact gtgtggtggc
 151 acagcagcaa cgggctgagc tcggacctgt cctgtcttcc agatcacagc
 201 ttt

365

626627-626.Seq Length: 192 July 3, 1990 15:27 Check: 1506 ..

1 CTGAGCTCGG ACCTGTCTTG TCTTCCAGAT CACAGCGTTC GCCCGCTCC
 51 AGTTGCAGGC CGCTTCCAGC CTGTGCTCGA CCCCTCCAGG GTACATCTTG
 101 ACTTTGAATA TGGTGTAAIT GATGTACAA GATAGTGC AAAGCCTATA
 151 CGGGTACTCG ACCATGCACC TGGGTGTAAI CCAGGACCGA AG

365

626627-627.Seq Length: 301 July 3, 1990 15:31 Check: 6567 ..

1 AGGCCGCTGG ACAAGGCTGG CAGGGTCTGG AAAGAACAGG GAAGGACCTG
 51 CCACTGTGTG TGGACAGCAG CAACGGGCTG AGCTGGAGC TGTCCCTGTG
 101 TTCCAGATCA CAGCGTTCGC CCCGCGTCCA GTTGACGGCC GCTTCCAGCC
 151 TGTGCTCGAC CCTTCCACAG TACATCTTGA CTTTGAATAT GGTGTAAITG
 201 ATGGTACAA GATAGTGC CAAGCCTATAC GGGTAGTCCA CCAATGCACCT
 251 GGGTGTAAIT CTGGGACCGG AGCCGACCGG AGAGTATGTG GCTTCCGATA
 301 T

365

626627-591.Seq Length: 292 July 3, 1990 15:24 Check: 2620 ..

1 TATCGAATTC CTGAGCCCC CGCAAAGAGC GTGTGTGGCC CGGTATATTG
 51 CTTCACCTCC AGCCCCGTGG TGGTGGGAAC GACCGACAGT GCGCGCTTAC
 101 CTACAGCTGG GGTGCAAAATG ATACGGATGT TTCTGCTCTT ACACAACACC
 151 AGGCCACCGC TGGGCAATTG GTTCGGTGTG ACCTGGATGA ACTCAACTGG
 201 ATTACCAAAA GTGTGCGGAC GCCCCCTTGT GTATCTGGAG GGGTGGGCAA
 251 CAACACCTTG CTCTGCCCCA CTGATTGCTT CGCAAAATAT GG

365

626627-U.Seq Length: 225 July 2, 1990 11:34 Check: 6325 ..

1 TAATACGACT CACTATAGGG CGAATGGGT ACCGGGCCCC CCTCTGAGGT
 51 CGACCGTATC GATAAGCTTG ATATCGAATT CCTGCAGGCC CGGCAAGAG
 101 CGTGTGTGGC CGGTATATT GTTCACTCC CAGCCCCGTG GTGGTGGGAA
 151 CGACCGACAG GTGCGGCGCT ACCTACAGCT GGGGTGCAAA TGATACGGAT
 201 GTCTTGTCTT TTAACAACAC CAGGC

365
AB

626627 organization HIV

Hcv.Seq x 626627-591.Seq July 6, 1990 09:29 ..

```
144 CCCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT 193
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
19  CCCGCAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCAGCCCCGT 68
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
194 GGTGGTGGGAACGACCGACAGGTGCGGGCGCGCCACCTACAGCTGGGGTG 243
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
69 GGTGGTGGGAACGACCGACA...GTGCGCGCCTACCTACAGCTGGGGTG 114
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
244 AAAATGATACGGACGCTTCGTCTTA.ACAATACCGAGGCCACCGCTGGG 292
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
115 CAAATGATACGGATGTCTTCGTCTTAACAACACCGAGGCCACCGCTGGG 164
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
293 CAATTGGGTTGGGTTGTACCTGGATGAACTCAGTGGATTACCAAGGTGT 342
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
165 CAATTGGGTTGGGTTGTACCTGGATGAACTCAGTGGATTACCAAGGTGT 214
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
343 GCGGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 392
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
215 GCGGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCTC 263
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
393 TGCCCCACGATTGCTTCCGCAAGCATCCG 422
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
264 TGCCCCACGATTGCTT.CGCAAAATATCGG 292
```

compare
confirm

Hcv.Seq x 626627-Nybc.Seq July 6, 1990 09:30 ..

```
145 CCGCGAAGAGTGTGTGTGGTCCGGTATATTGCTTCACTCCCAGCCCCGT 194
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
1  CCGCAAGAGCGTGTGTGGCCCGGTATATTGCTTCACTCCCAGCCCCGT 50
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
195 GTGGTGGGAACGACCGACAGGTGCGGGCGCGCCACCTACAGCTGGGGTG 244
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
51 GTGGTGGGAACGACCGACA...GTGCGCGCCTACCTACAGCTGGGGTG 100
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
245 AAATGATACGGACGCTTCGTCTTAACAATACCGAGGCCACCGCTGGGCA 294
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
101 AAATGATACGRATGTCTTCGTCTTAACAACACCGAGGCCACCGCTGGGCA 150
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
295 ATTGGTTGGGTTGTACCTGGATGAACTCAGTGGATTACCAAGGTGTGC 344
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
151 ATTGATTGGGTTGTACCTGGATGAACTCAGTGGATTACCAAGGTGTGC 200
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
345 GGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCAC 394
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
201 GGAGCGCCTCCTTGTGTATCGGAGGGGGCGGGCAACAACCCCTGCTC 250
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
395 CCCCAGTGAATTGCTTCCGCAAGCATCCGGAGCCACATACTCTCGGTCG 444
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
251 CCCCAGTGAATTGCTTCCNTAAATANCYRAANGCTAYANACTNICAAGNN 300
      ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
445 G 445
      |
301 G 301
```

7.6.90

HCV organization 2/626627 done

0000

Hcv.Seq x 626627-627.Seq July 6, 1990 08:20 ..

```

421 CCGACGCCACATACTCTCGGTGCGGCTCCGGTCCCTGGATCAGACCCAGG 470
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
996 CCGAAGCCACATACTCTCGGTGCGGCTCCGGTCCAGGATTACACCCAGG 942
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
471 TGCCTGGTCGACTACCCGTATAGGCTTTGGCATTATCCTTGTACCATCAA 520
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
946 TGCATGGTCGACTACCCGTATAGGCTTTGGCACTATCCTTGTACCATCAA 892
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
521 CTACACCATATTTAAAAATCAGGATGTACGTGGGAGGGGTCGAACACAGGC 570
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
896 TTACACCATATTCAAAGTCAGGATGTACGTGGGAGGGGTCGAACACAGGC 842
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
571 TGGAAAGCTGCCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGGAAAGAC 620
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
846 TGGAAAGCGGCTGCAACTGGACGCGGGGCGAACGTTGCGATCTGGAAAGAC 792
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
621 AGGGACAGGTCGGAGCTCAGCCCGTTACTGCTGACCACTACACAGTGGCA 670
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
296 AGGGACAGGTCGGAGCTCAGCCCGTTGCTGCTGTCACACACAGTGGCA 748
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
671 GGTECTCCCGTGTTCCTTCACAACCCCTACAGCCCTGTCCACCGGCT 718
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
247 GGTCCTTCGTTCTTTTACAGACCCCTGCCAGCCCTGTCCACCGGCT 700
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

```

missing
confirm

cc

Hcv.Seq x 626627-590.Seq July 6, 1990 09:27 ..

```

601 AACGTTTCGATCTGGAAGACAGGGACAGGTCGGAGCTCAGCCCGTTACTG 650
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
1000 aaagctgtagctctggaagacaaggacaggtccaggtccagccattgctg 952
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
651 CTGACCACTACACAGTGGCAGGTCCTCCCGTGTTCCTTCACAACCCCTAC 699
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
951 ctgtgcccccacacagtgagcaggtccctccgtggtctttcagacccctgc 902
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
700 CAGCCTTGTCCACCGGCTCATCCACCTCCACAGAACATTGTGG 744
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
301 cagccttgctccacccggctcatccacctccacagaaacattgagg 852
    ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

```

corrected entire seq of HCV

-pkc = confirmed A w/HCV

Computer Assembly (cont'd)

21F1 - F seq

21F1 - U #1-272
21F1 - 675 #137-137
21F1 - 674 #275-39
21F1 - r #56-1

Δ 198 del A, +T
add GG
del AC
add t

HCV pair

5600-6600

524bp
95.029 / 3

628629 - F seq

628629 - U #1→225
628629 - 628 #96-217
628629 - r #1→2052

HCV pair

850-1231

382 bp home
94.76 / 5mi

626627 - F seq

626627 - U #1→225
626627 - 591 155-end
626627 - 627 #1→297
626627 - 590 #1→85

HCV pair

144-900

601 bp home
94.509 / 5mi

w/o Computer Assembly of Sequence Information

Notes on organization and Bestfit Analysis

571623 - 10F seq.

add sequence info on to end of already established file
pair w/ HCV

3300 - 3600

243 bp hc
97.1% sim

62126 - 5F seq

from 5U

121 - del A

150 - W = A

129 - add del G

207 - add A

HCV pair

5959 - 6200

182 bp hc
97.2% sim

628652 - 2F seq

from 2U

no A

HCV pair

852 - 959

108 bp hc
98.148

617652 - 4F seq

from 571623 - 4U

94 del C

168 T → C

HCV pair

789 - 960

172 bp hc
97.093

7/19/90

ALF sequencing

9 JULY 90

clones

1	654 687	250bp	#6	universal	5ug
2	654 687	250bp	#4	reverse	10ul
3	654 687	250bp	#1	universal	10ul
4	654 687	250bp	#1	reverse	10ul
5	654 687	120bp	#6	universal	6ul
6	654 687	120bp	#6	reverse	6ul
7	690 691	#8	PCR, btm	universal	6.8ul
8	690 691	#8		reverse	6.8ul
9	691 693	#1	PCR	universal	5ul
10	691 693	#1		reverse	5ul

Derivative Templates

Sequence with new kit (6/27/90)

deionized gel formula with Jeffs BioRad Resin

Results

no good sequence info.

all overlapping peaks (clones 1, 2, and 9)

or "ASE 1002" error

peak width cannot be computed (remainder)

Probably not problems with kit as John has used it successfully

7.11

000074

35S sequencing

to obtain information on plasmids
- problems with AIF

To sequence:

					DNA	H ₂ O
1	654687	top #1	410 ng/lul	Universal	12.4	6
2	"			Reverse	12.4	6
3	654687	btm #6	820 ng/lul	Universal	6.1	12
4	"			Reverse	6.1	12
5	690691	#8	730 ng/lul	Universal	6.8	5.2
6	"			Reverse	6.8	5.2
7	691693	#1	1.04 ng/lul	Universal	5.1	13
8	"			Reverse	5	13
9	PCR 2 #2		465 ng/lul	Universal	10.7	7.3
10	"			Reverse	10.7	7.3
11	PCR 2 #7		740 ng/lul	Universal	6.7	11.24
12	"			Reverse	6.7	11.24

use Pharmacia Sequencing kit (Cydric)

includes universal primer

use reverse primer F12 from AIF kit.

8.1 sequencing gel

35S long

1
2
3
4
5
6
7
8
9

1/2

10

11

12

=

1 repeated

35S short

1
2
3
4
5

1/2

6

7

8

9

10

11

12

cracked gel
from plates

Results

Sequences OK

except PCR 2 #2 or #7.

miniprep Bacteria in From Genevive - NYBC

clones	10-12 primers	#17	tissue 298	392 bp core
	10-12 primers	#43	SA cDNA	392 bp core
	15-17 primers	#26	tissue 19-16 ¹⁹⁻¹²	617 bp env
	15-18 primers	#31	tissue 14-13 ¹⁴⁻¹²	151 bp env
	15-18 primers	#51	cDNA 14-12	151 bp env

inoculate cultures: max prep.

PHOTOMETRY/DNASCAN4U

07/11/90 11:18

ID	ABS		
260.0nm	0.425A	3	2.125ug/L
260.0nm	0.027A	51	135ng/L
260.0nm	0.057A	17	285ng/L
260.0nm	0.157A	43	785ng/L
260.0nm	0.391A	61	1.955ug/L
260.0nm	0.076A	26	380ng/L

Primer Locations:

[illegible]

12 JULY 90 - ALF sequencing

#	clone	primer	Denature	
			DNA	H ₂ O
1	654 687 *6	universal	6	4
2	654 687 *6	reverse	6	4
3	690 694 *9	universal	2.5	7.5
4	690 694 *9	reverse	2.5	7.5
5	690 694 *11A	universal	6.4	3.6
6	690 694 *11A	reverse	6.4	3.6
7	690 691 *21B	universal	2.5	7.5
8	690 691 *21B	reverse	2.5	7.5

Set up on ALF

old model plate - new copier (used w before)
 used new "non prototype" kit

results:

"peaks cannot be computed"

shown on 1-2 & 5-8 clones

peaks 3-4 were computed

but data is all overlapping

Potential problem shooting:

try new glass plate

new copier

kit was used previously by John with positive re

7/12

³⁵S sequencing of plasmids

ds procedure → CYNDIES kit
³⁵S of 7/10

	clone	primer	DNA	H ₂ O
1	10.12 #17	590	18	
2	10.12 #17	591	18	
3	10.12 #17	693	18	
4	10.12 #17	694	18	
5	10.12 #43	590	6.3	11.7
6	10.12 #43	591	6.3	11.7
7	10.12 #43	693	6.3	11.7
8	10.12 #43	694	6.3	11.7
9	15.17 #26	590	13.15	4.85
10	15.17 #26	591	13.15	4.85
11	15.18 #31	590	2.35	15.65
12	15.18 #31	591	2.35	15.65

long gel

1
2
3
4
5
6
7
8

12

9

10

11

12

-

2

3

4

old samples

short gel

3

4

5

6

-

1

2

3

4

5

6

7

8

9

10

11

12

ad samples

4 → ran a little
 12

Results. primers 653 + 654 did not work - did not arrive?

7/16	New Round	ALF
------	-----------	-----

1. 628 629 - universal
2. 628 629 - reverse
3. 628 629 - 702
4. 628 629 - 703
5. 690 694 #9 - universal
6. 690 694 #9 - reverse
7. 15 17 #26 - universal
8. 15 17 #26 - reverse
9. 15 17 #26 - 702
10. 15 17 #26 - 703

$$2\text{K}_2\text{S}_2\text{O}_8 + \text{H}_2\text{O} \rightarrow 2\text{K}_2\text{SO}_4 + \text{H}_2\text{SO}_4$$

1001 DNA

set up of ALF

new loader

changed to second set of non prototype kit ingredients
sequence reactions done in water bath at 37°

16 Jul 90. AEF

1	628629	processed data but slightly overlapping
2	r	overlapping data
3	702] could not compute peak system error
4	703	
Export	5 690694 - 90	worked! 300bp sequence
6	9r	GIS did not resolve well.
7	15.17 - 0] some gaps - problems with A peaks trend = 0.00
8	15.17 - r	
9	15.17 - 702	processed, but trend = 0.00
10	15.17 - 703	system error

only one clone worked, but results seem more encouraging than previous failed runs.

7/12 ^{35S} sequencing

To complete sequences of remaining plasmids constructed.

^{35S} sequence : use Cynetics AutoRead kit.

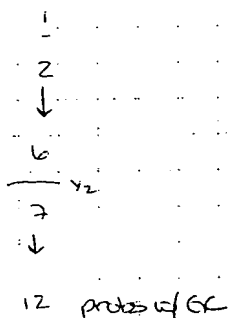
^{35S} from 7/10.

set up template denaturation O/N : sequence, run gels next AM.

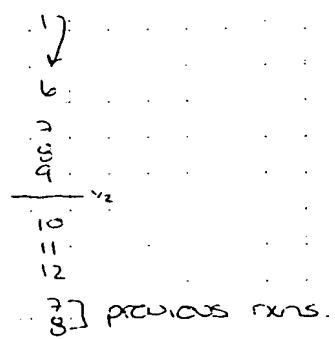
#	clone	primer	DNA	H ₂ O
1	690.694 #9	universal	2.5	15.5
2	690.694 #9	reverse	2.5	15.5
3	690.694 #11A	universal	6.5	11.5
4	690.694 #11A	reverse	6.5	11.5
5	690.691 #21B	universal	2.5	15.5
6	690.691 #21B	reverse	2.5	15.5
7	10.12 #17	690	18	
8	10.12 #17	691	18	
9	10.12 #43	690	6.4	11.6
10	10.12 #43	691	6.4	11.6
11	15.18 #51	702	18	
12	15.18 #51	703	18	

gels

long



short



NOTES on 10.12 capsid gene cloning from NYBC.

re FAX of July 11. from NYBC

Sequence shows limited homology with Japanese primers - most homology later determined to not be a correct clone.

000077

7/19/90

19 JUL 90 ALF

ALF sequencing run

done with Mark Freeman
Pharmacia NJ.

clones:

1	PUC18	universal	} kit controls
2	PUC18	reverse	
3	M13	universal	
4	690691	ISE universal	} undies 3S primers
5	690691	ISE reverse	
6	15-17	*26 702	
7	15-17	*26 703	
8	PUC18	universal	
9	PUC18	reverse	
10	M13	universal	

1200 volts 400S
40mA 10 clones
36W
45°-40°
3mW

Denaturation of Templates

A. PUC18 control 2* universal 500ng/ul =
2* reverse 10ul for Sug

B. 690691 ISE

402ng/ul - use 10ul

did 10ul DNA

+ 3ul 2M NaOH

C. 15-17 = 380ng/ul - use 10ul

+ 10ul + NaAC + EDTA according to previous pen
ETOH ppt

Did not work w/ 70% → sequence as usual

ALF run did not work for ss or ds templates.

problems w/ ds runs; NaOH denaturation?
unlikely is same procedure for 3SS.

7/20/90

20 JUL 90 ALF

repeat of ALF sequencing
for ss M13 runs only.
check for machine working

1	} S12 w/ 29 kit	M13 universal primer	
2		M13 [extension buffer] w/ anneal buffer	
3		M13 + T ₇ from 3'S kit	
4		M13	
5		M13	

6	} MF w/ 29 kit (non prototype)	M13 universal primer	
7		M13 w/o extension buffer. 1cm	
8		M13 w/o " + H ₂ O	
9		M13 + T ₇ from 3'S kit	
10		M13	

Results = Run worked! NO machine / software problems

Exhibit 4, page 29

690694-92u.Seq x 690694-9u.Seq July 19, 1990 07:00 ..

269.

14 TCTAGAGGATCCCCGTCCTGCCCCGCGCCGACGTGCCCTTGGGGATAGGC 63
1 TCTAGAGGATCCCCGTCCTGCCCCGCGCCGACGTGCCCTTGGGGATAGGC 50
64 TGACGTCTACCTCGAGGTTGCGACCGCTCGGAAGTCTTCTTCGTCGCGCG 113
51 TGACGTCTACCTCGAGGTTGCGACCGCTCGGAAGTCTTCTTCGTCGCGCG 109
114 CACACCCAATCTAGGGCCCCCTGCGCGGCAACAAGTAACTCCACCAACGA 163
101 CACACCCAATCTAGGGCCCCCTGCGCGGCAACAAGTAACTCCACCAACGA 150
164 TCTGACCGCCACCCGGGAACCTGACGTCTTGTGGGCGACGGTTGGTGTGA 213
151 TCTGACCGCCA.CCGGGAACTTGACGtCtGtGGGCGACGGTTGGTGTGA 199
214 CGTTTGGTTTTTCTTTGAGGTTTGGGAATGTGC 246
200 CGTT..GGTTTTCTTTGAGG.TTGGGAATGTGC 229

probs
w/9u

690694-92u.Seq x 690694-92r.Seq July 19, 1990 08:02 ..

92u/
92r=α

eqs α
better

46 CGTGCCTTGGGGATAGGCTGACGTCTACCTCGAGGTTGCGACCGCTCGGA 95
246 CGTGCCTTGGGGATAGGCTGACGTCTACCTCGAGGTTGCGACCGCTCGGA 197
96 AGTCTTCTCTGTCGCGCGCACACCCAATCTAGGGCCCCCTGCGCGGCAACA 145
196 AGTCTTCTCTGTCGCGCGCACACCCAATCTAGGGCCCCCTGCGCGGCAACA 147
146 AGTAAACTCCACCAACGATCTGAC.GCCACCCGGGAACCTTGACGTCTTGT 195
146 AGTAAACTCCACCAACGATCTGACCGCCACCCGGGAACCTTGACGTCTTGT 97
193 GGGCGACGGTTGGTGTACGTTTGGTTTTTCTTTGAGGTTTGGGAAT 242
93 GGGCGACGGTTGGTGTACGTTTGGTTTTTCTTTGAGGTTTGGGAAT 50

690694-11au.Seq x 690694-11ar.Seq July 19, 1990 07:02 ..

31 ATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 80
238 ATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 189
81 GGACGTCAAGTTCCTGGGTGGCGGTCAGATCGTTGGTGGAGTTTACTTGT 130
188 GGACGTCAAGTTCCTGGGTGGCGGTCAG.TCGTTGGTGGAGTTTACTTGT 140
131 TGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 180
139 TGCCGCGCAGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 90
181 GAGCGGTTCGCAACCTCGAGGTAGACGTACGCTATCCCAAGGCACGTG 230
89 GAGCGGTTCGCAACCTCGAGGTAGACGTACGCTATCCCAAGGCACGTG 40
231 GCCCGAGG 238
39 GCCCGAGG 32

see next page

Sequence Organization ALF - 355 runs

000078

690694

REFORMAT of: 690694-9u.Seq check: 7228 From: 1 To: 287 July 17, 1990 10:42
(No documentation)

690694-9u.Seq Length: 287 July 17, 1990 10:42 Check: 7228 ..

1 TCTAGAGGAT CCCCCTCTCT CCCCCTGGGCC GACGTGCTT GGGGATAGGG
51 TGACGTCTAC CTCCAGGTTG CGACCGCTCG GAAGTCTTCC TCGTCGCGCG
101 CACACCCAAT CTAGGGCCCC TCGCGGCGAA CAAGTAAACT CCACCAACGA
151 TCTGACCGCC ACCGGGAAGT TGACGTCTCT TGGCGACGG TTGGTGTAC
201 GTTGGTTTTC TTGAGGTTG GGAATGCT CATGGGTAC AGCTCGAATC
251 gtaacgctc aagcggttcc tctgtgaaatg ttaaaa

690694-92u.Seq Length: 246 July 18, 1990 14:30 Check: 298 ..

1 CCTGCAGGTC GACTCTAGAG GATCCCCGTC CTGCCCTCGG GCCGACGTGC
51 CTTGGGGATA GGCTGACGTC TACCTCGAGG TTGCGACCGC TCGGAAGTCT
101 TCCTCGTCGC GCGCACACCC AATCTAGGGC CCTGCGCGG CAACAAGTAA
151 ACTCCACCAA CGATCTGACC GCCACCGGG AACTTIGACGT CCGTGGGGCG
201 ACGGTITGGT TTACGTTTGG TTTTCTTTG AGGTTTGGGA ATGTGC

690694-92r.Seq Length: 220 July 18, 1990 14:34 Check: 5194 ..

1 CGAGCTCGGT ACCCATGAGC ACGATTCCCA AACCTCAAAG AAAAACCAAA
51 CGTAACACCA ACCGTCGCCC ACAGGACGTC AAGTCCCCGG GTGGCGGTCA
101 GATCGTTGGT GGAGTTTACT TGTTCGCGG CAGGGGGCCCT AGATTGGGTG
151 TCGCGCGGAC GAGGAAGACT TCCGAGCGGT CGCAACCTCG AGGTAGACGT
201 CAGCCTATCC CCAAGGCACG

690694-113u.Seq Length: 238 July 18, 1990 14:40 Check: 9399 ..

1 GGTCGACGCT AGAGGATCCC CATGAGCAGC ATTCCCAAAC CTCAAAGAAA
51 AACCAAACGT AACACCAACC GTGCGCCACA GGACGICAGG TTCCCGGGTG
101 GCGGTCAGAT CGTGGTGGG GTTACTTGT TGCCGCGCAG GGGCCCTAGA
151 TTGGGTGTGC GCGCGACGAG GAAGACTTCC GAGCGGTTCG AACCTCGAGG
201 TAGACGTCAG CCTATCCCCA AGGCACGTCG GCCCGAGG

690694-114r.Seq Length: 225 July 18, 1990 14:44 Check: 3275 ..

1 GCTCGGTACC CGTCTGCCC TGGGCGGAC GTGCTTGGG CATAGGCTGA
51 CGTCTAGCTC GAGGTTGTA CGCTCGGAA GTTCTCTCG TCGCGCGAC
101 ACCCAATCTA GGGCCCTGC GCGGCAACAA GTAACTCCA CCAACGACTG
151 ACCGCCACCC GGGAACTTGA CGTCTCTCG GCGACGTTG GTGTTACGTI
201 TGGTTTCTT TTGAGGTTTG GGAAT

690694 - continued

690694-11au.Seq x 690694-92u.Seq July 19, 1990 07:05 ..

seq of
92u=oc
match to
11A

```
31 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 80
   |||||||
234 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 185
   |||||||
81 GGACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGT 130
   |||||||
184 GGACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGT 135
   |||||||
131 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 180
   |||||||
134 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 85
   |||||||
181 GAGCGGTGCGCAACCTCGAGGTAGACGTGAGCCATATCCCCAAGGCACGTGG 230
   |||||||
84 GAGCGGTGCGCAACCTCGAGGTAGACGTGAGCCATATCCCCAAGGCACGTGG 35
   |||||||
231 GCGCGAGG 238
   |||||||
34 GCGCGAGG 27
```

Hcv-Core.Seq x 690694-92u.Seq July 19, 1990 07:03 ..

= only
changes
with HCV

→ 690

```
10 attccccaaacctcaaaagaaaaaccaaaccgtaacacccaaccgtcgcacaca 59
   |||||||
376 ATTCCTCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCACACA 327
   |||||||
60 ggacgtcaagttcccggtggcggtgagatcggtgggtgggtttacttgt 109
   |||||||
326 GGACGTCAAGTTCCCGGGTGGCGGTGAGATCGTTGGTGGAGTTTACTTGT 277
   |||||||
110 tgccgcgccagggggccctagattgggtgtgcgcgcgacgagggaaacttcc 159
   |||||||
276 TGCCGCGCAGGGGGCCCTAGATTGGGTGTGCGCGCGACGAGGAAGACTTCC 227
   |||||||
160 gagcggtcgcaacctcgaggtgagcgtagcctatccccaaagggtgcgtcg 209
   |||||||
226 GAGCGGTGCGCAACCTCGAGGTAGACGTGAGCCATATCCCCAAGGCACGTGG 177
   |||||||
210 gcccggggggcaggacctgg 228
   |||||||
176 GCGCGAGGCGAGGACGGGG 158
```

Hcv-Core.Seq x 690694-9u.Seq July 17, 1990 18:06 ..

690

```
1 atgagcagcattccccaaacctcaaaagaaaaaccaaaccgtaacacccaaccg 50
   |||||||
226 ATGAGCAGC.ATTCCC.AACCTCAAACAAAAA...CCAACTTAACACCAACCG 281
   |||||||
51 tgccccacaggagcgtcaagttcccggtggcggtgagatcggtgggtgggt 100
   |||||||
280 TGCCCCAGCAGGAGCTCAAGTTCCC.GGTGGCGGTGAGATCGTTGGTGGAG 332
   |||||||
101 ttacttgttgccgcgccagggggccctagattgggtgagcgcgcgagcgag 150
   |||||||
231 TTTACTTGTGTGCGCGCGAGGGGGCCCTAGATTGGGTGTGCGCGCGAGCGAG 182
   |||||||
151 aagacttcggcggtgagcaacctcgaggtgagcgtagcctatccccaa 200
   |||||||
181 AAGACTTCCGAGCGGTGCAACCTCGAGGTAGACGTGAGCCATATCCCCAA 132
   |||||||
201 ggtggtcgggcccgaggacctgg 226
   |||||||
131 GGCACGTGCGCCGAGGGGAGGACGGGG 104
```

probably
this 9u seq
see previous
page

691693 clone

only one isolated - sequenced = #1

00007

691693-1u.Seq Length: 280 July 17, 1990 13:13 Check: 8434

1 TTGCATGCTT GCAGGTCGAC TCTAGAGGAT CCCCATTCAC GTCCGCCCGA
51 GGGCAGGACC TGGGCTCAGC CCGGTACCTT TGGCCCTCT ATGGCAATGA
101 GGGTTGCGGG TGGGCGGGAT GGTCTCTGTC TCCCCGTGGC TCTCGGCTA
151 GCTGGGGCCC CACAGACCCC CGGCGTAGGT CGGCAATTT [→]GGGTACCGAG
201 CTGGAATTTC TAATCATGTC ATAGCTGTTT CCTGTGTGAA ATTGTTATCC
251 GCACACAATT CCACACAACA TACGAGCCGG

691693-1r.Seq Length: 195 July 17, 1990 13:16 Check: 400

1 TTGAGGCTCG [←]GTACCCAAAC GCGACCTACG CCGGGGGTCT GTGGGGCCCC
51 AGCTAGGCCG AGAGCCACGG GGAGACAGGA GCCATCCCGC CCACCCGCAA
101 CCTCATTTGC CATAGAGGGG CCAAGGGTAC CCGGCGCTGA GCGAGGTCTT
151 GGGCAGGGG CGAGGTGCTT [←]GGGATCCTC TAGAGTCGAC CTGCA

691693-1u.Seq x 691693-1r.Seq July 17, 1990 13:05

10 TGCAGGTCGACTCTAGAGGATCCCA [←]TCAGGTCGSCCC [←]GGCAGGAC 59
240 TGCAGGTCGACTCTAGAGGATCCCA [←]AGCAGCTCGGCCCG [←]AGGCGAGGAC 231
60 CTGGGCTCA [←]CGGGGTACCC [←]TGGCCCCCTCTATGGCAATGAGGGTTGC 107
230 CTGGGCTCA [←]CGGGGTACCC [←]TGGCCCCCTCTATGGCAATGAGGGTTGC 182
108 GGGTGGGCGGGATGGCTCTCTCTCCCCGTGGCTCTCGGCTAGCTGGGG 157
181 GGGTGGGCGGGATGGCTCTCTCTCCCCGTGGCTCTCGGCTAGCTGGGG 130
158 CCCCACAGACCCCCGGGTAGGTCGCGCAATT [←]GGGTACCGAGCTCGAA 206
131 CCCCACAGACCCCCGGGTAGGTCGCG... [←]TTGGGTACCGAGCTCGAA 84

check on seq films.

with corrections to
691693-1u.

Hcv-Core.Seq x 691693-1u.Seq July 17, 1990 18:07

172 cctcgggtagacgtcagcctatccccgaggtagcgtcggcccgaggagag 221
8 CCTGCAGGTCGACTCTAGAGGATCCCA [←]ATTC [←]ACGTCGGCCCCGAGGGCAG 51
222 gacctgggctcagcccggtacccttggcccccctatggcaatgagggct 271
57 GACCTGGGCTCAG [←]CCGGTACCC [←]TGGCCCCCTCTATGGCAATGAGGGIT 107
272 gagggtggggatgactcctgtctccccgtggctctcagccctattgag 321
106 GCGGGTGGGCGGGATGGCTCTCTCTCCCCGTGGCTCTCGGCTAGCTGG 157
322 gggcccgaggacccccgggtaggtaggggatt [←]ggggtagggtaggg 157
156 GGGCCACAGACCCCCGGGTAGGTCGCGCAATT [←]GGGTACCGAGCTCGAA 206

= confirmed
changes w/ HCV

clone is not full
length 693-691
stops past the
694 ntiles

NOTE → 49 → CA Δ is
confirmed

1518-51702.Seq x 1518-51nybc.Seq July 24, 1990 19:09 ..

```

      15
1' GGTTCATCGATACCCTTACGTGCGGCTTCG56CCACCTCATGGGGTACATAC 50
   |||
4 GGTTCATCGATACCCTTACGTGCGGCTTCG56CACCTCATGGGGTACATAC 53
   |||
51 CGCTCGTCGGCGCCCCCTCTTGGAGGCCGTGCCAGGGCCCTGGCGCATGGC 100
   |||
54 CGCTCGTCGGCGCCCCCTCTTGGAGGCCGTGCCAGGGCCCTGGTGCATGGY 103
   |||
101 GTCCGGGTTCTGGAAGACGGCGTGAACCTATGCAACAGGGAACTTCCCAG 150
   |||
104 GTCCGGGTTCTGGAAGACGGCGTGGGCTATGCGACAGGGGGCCTTCCCAG 153
   |||
      48
151 TTGCTCTTTCTCTATC 166
   |||
154 TTGCTCTTTCTCTATC 169
   |||
```

1518-51f.Seq x 1518-51703.Seq July 26, 1990 15:30 ..

```

37 TTCGCGCACCTCATGGGGTACATACCGCTCGTCGGCGCCCCCTCTTGGAGG 86
   |||
220 TTCGCGCACCTCATGGGGTACATACCGCTCGTCGG. GCCCCTCTTGGAG. 173
   |||
87 CCGTGCCAGGGCCCTGGCGCATGGCGTCCGGGTTCTGGAAGACGGCGTGA 136
   |||
172 .CGCTCCAGGGCCCTGG. CATGGCGTCCGGGTTCTGGAAGACGGCGTGA 125
   |||
137 ACTATGCAACAGGGAA. CCTTCCCA. GTTGCTCTTTCTCTATCTGGGGGA 184
   |||
124 ACTATGCAACAGGGAA/CCTTCCCA/GTTGCTCTTTCTCTATCTGGGGGA 75
   |||
185 TCCACTAGTTCTAGAGCGGCCGCCACCGC. GTGGAGC 220
   |||
74 TCCACTAGTTCTAGAGCGCGCACCGCGGTGGAGC 38
   |||
```

use 702: NYBC sequence 1st = F version.
then compare Δ with 1518-51703

clone 31 results → NYBC
only homology = w/ primer
see FAX of July 11.

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